

Other example of fusion:

Construct CST-04: Fusion of the *Neisseria meningitidis* CMP-Neu5Ac synthetase (GenBank #U60146) with Cst-I from *Campylobacter jejuni* (GenBank #BD134499). This construct also includes a Gly-Gly-Gly-Ile linker between the fusion partners and a 6-His tag at the C-terminus.

Figure 1: DNA sequence of the fusion gene in CST-04

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ATGGAAAAACAAATATTGCGGTTATACTTGCAGCCAAAACTCCAAAGGATTGCCATTAAAAA
ATCTCCGGAAAATGAATGGCATATCATTACTTGGTCATAACAATTAAATGCTGCTATATCATCAAA
GTGTTTGACCGCATAATTGTTCGACTGATGGCGGGTTAATTGCAAGAAGCTAAAATTTC
GGTGTGAAGTCGCTCACGCCCTGCAGAGCTGGCCTCCGATACAGCCAGCTCTATTCAGGTG
TAATACATGCTTAGAAACAATTGGCAGTAATTCCGGCACAGTAACCCATTACAACCAACCAG
TCCATTACGCACAGGGCTCATATTGTGAAGCTTTCTCTATTGATGAGAAAATAAAAGGA
TCCGTTGTCTCTGCATGCCAATGGAGCATCCACTAAAAACCCCTGCTCAAATCAATAATG
GCGAATATGCCCATGCGCCATCTAACGCGATTGGAGCAGCCTCGCCAACAATTACCTCAGGC
ATTTAGGCCTAATGGTCAATTACATTAATGATACTGCTTCATAATTGCAAATAATTGTTT
TTATCGCTCAACCAAACCTTATATTATGTCTCATCAAGACTCTATCGATATTGATACTGAGC
TTGATTACAACAGGCAGAAAACATTCTTAATCACAAGGAAAGCGGTGGCGGAATTCTGACAAG
GACTAGAATGGAAAATGAACTCATTGTTAGTAAAATATGCAAAATATAATCATAGCAGGAAAT
GGACCTAGCCTAAAAATATTAATTATAAAAGACTGCTAGAGAATATGATGTTTTAGGTGTA
ACCAGTTTATTTGAAGATAAGTATTATTAGGAAAAAGATTAAGCAGTATTTTTAATCC
TGGTGTCTTTTACAACAGTATCACACTGCAAAACAACCTTACTAAAAATGAGTATGAAATA
AAAAATATTTTGCTCTACATTAAATTACCTTTATTGAAAGCAATGATTTTACATCAAT
TTTATAATTTCCTCCGATGCAAAACTTGGCTATGAAGTTATTGAAAACCTTAAAGAATTTA
TGCTTATATAAAATACAATGAAATTATTCAATAAAAGATTACTCGGGCGTCTATATGTGT
GCAATTGCTATTGCATTAGGATATAAAACCATCTATTATGTGGCATTGATTTTATGAAGGAG
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ATTGACTGATAATACTCCTGGCGTAAGTTTATAAAATCAACTTAAAGCTGATAATAAAATT
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TTGCGGTATTAAAAACAAACCACTCAACGAGCTAAAGCAAGAATCCAAAACCATCTATCCTA
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ATAATTAAAGTATCGTTATTCACATAACAAAGAACAAAAGGCTTATAAATTAAAGTAAAGA
AAAATCCAAATTAGCTTACCTCCTTAGAAAACCTTACCTGATTATAATGAAGCTTAAAGA
AAAAGAATGTTTACTTATAAAATTAGGAGAAGAATTATAAAAGCTGGTAAGAATTGGTATGGG
GAGGGGTATATCAAATTATTCACAAAGATGTTCTAGGTTGAAGAGAGAGTTGAGAAAGGGG
AACATCACCACCATCACCACCAATGA
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Figure 2: Amino acid sequence of the fusion protein in CST-04.

EXHIBIT C

MEKQNIAVILARQNSKGLPLKNLRKMNGISLLGHTINAAISSKCFDRIIVSTDGGGLIAEEAKNF
 GVEVVLRPAELASDTASSISGVIHALETIGSGTVTLLQPTSLRTGAHIREAFSLFDEKIKG
 SVSACPMEEHPLKTLLQINNGEYAPMRHLSDEQPRQQLPQAFRPNGAIYINDTASLIANNCF
 FIAPTKLYIMSHQDSIDIDTELDLQQAENILNHKESGGGILTRRMENELIVSKNMQNIIAGN
 GPSLKNINYKRLPREYDVFCRNQFYFEDKYYLGKKIKAVFFNPGVFLQQYHTAKQLILKNEYEI
 KNIFCSTFNLPFIIESNDFLHQFYNFDPDAKLGYEVIENLKEFYAYIKYNEIYFNKRITSGVYMC
 AIAIALGYKTIYLCGIDFYEGDVIYPFEAMSTNIKTIFPGIKDFKPSNCHSKEYDIEALKLLKS
 IYKVNIYALCDDSI LANHFPLSINIINNNFTLENKHNNNSINDILLTDNTPGVSFYKNQLKADNKI
 MLNFYNILHSKDNLIKFLNKEIAVLKKQTQRACKARIQNHLSYKLQALIINSKSVLGFLSLPF
 IILSIVISHKQEOKAYKFKVKKNPNLALPPLETYPDYNEALKEKECTYKLGEFFIKAGKNWYG
 EGYIKFIFKDVPRLKREFEKGEHHHHH

Experimental evidence for activity of both fusion partners:

Table 1: Activity measured in the various fractions of an extract¹ of *E. coli* AD202/CST-04 using a direct assay for α -2,3-sialyltransferase² activity and a coupled assay³.

Fraction	Total activity Direct assay ² (mU) ⁴	Total activity Coupled assay ³ (mU)
15,000 rpm supernant	2,289.6	1,612.0
15,000 rpm pellet	3,877.2	7,509.6
55,000 rpm supernatant	134.8	177.2
55,000 rpm pellet	895.2	897.2

¹ CST-04 was transformed in *Escherichia coli* AD202. A 200 mL culture was grown at 37°C and induced with 0.5 mM IPTG. The culture was grown for a total of 24 h and the cell extract was made using an Emulsiflex homogenizer. The extract was centrifuged at 15,000 rpm and the resulting supernatant was centrifuged at 55,000 rpm.

² The direct assay for α -2,3-sialyltransferase activity was performed using 1 mM Lac-FEX, 0.2 mM CMP-Neu5Ac, 10 mM MgCl₂, and 50 mM Hepes pH 7.5.

³ The coupled assay for CMP-Neu5Ac synthetase and α -2,3-sialyltransferase activities was performed using 1 mM Lac-FEX, 3 mM CTP, 3 mM Neu5Ac, 0.2 mM DTT, 10 mM MgCl₂, and 50 mM Hepes pH 7.5. The reactions were performed at 37°C for 5 min.

⁴ A milli-unit (mU) of activity is defined as the amount of enzyme that converts one nanomol of product per minute.

Other example of fusion: CST-02

Construct CST-02: Fusion of the *Neisseria meningitidis* CMP-Neu5Ac synthetase (GenBank #U60146) with truncated Cst-I (328 aa) from *Campylobacter jejuni* (GenBank #BD134499). This construct also includes a Gly-Gly-Gly-Ile linker between the fusion partners and a 6-His tag at the C-terminus.

Figure 1: DNA sequence of the fusion gene in CST-02

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ATGGAAAAACAAATATTGCGGTTATACTTGCAGCAGAAACTCCAAAGGATTGCCATTAAAAA  
ATCTCCGGAAAATGAATGGCATATCATTACTTGGTCATACAATTAATGCTGCTATATCATCAAA  
GTGTTTGACCGATAATTGTTCGACTGATGGCGGGTTAATTGCAGAAGAAGCTAAAAATTTC  
GGTGTGAAAGTCGTCCTACGCCCTGCAGAGCTGGCCTCGATAAGCCAGCTCTATTTCAGGTG  
TAATACATGCTTAGAACAAATTGGCAGTAATTCCGGCACAGTAACCCATTACAACCAACCAG  
TCCATTACGCACAGGGCTCATATTGTGAAGCTTTCTATTGATGAGAAAATAAAGGA  
TCCGTTGCTCTGCATGCCAATGGAGCATCCACTAAAAACCCCTGCTCAAATCAATAATG  
GCGAATATGCCCATGCGCCATCTAAGCGATTGGAGCAGCCTGCCAACATTACCTCAGGC  
ATTAGGCCTAATGGTGCATTTACATTAATGATACTGCTTCACTAATTGCAAATAATTGTTT  
TTTATCGCTCCAACCAAATTATATTATGTCTCATCAAGACTCTATCGATATTGATACTGAGC  
TTGATTACAACAGGCAGAAAACATTCTTAATCACAAGGAAAGCGTGGCGGAATTCTGACAAG  
GACTAGAATGGAAAATGAACCTCATTGTTAGTAAAATATGCAAAATATAATCATAGCAGGAAAT  
GGACCTAGCCTAAAAAATTAAATTATAAAAGACTGCCTAGAGAATATGATGTTTAGGTGTA  
ACCAGTTTATTTGAAGATAAGTATTATTAGGAAAAAAGATTAAAGCAGTATTTTAATCC  
TGGTGTCTTTACAACAGTATCACACTGCACAAACTTATACTAAAAATGAGTATGAAATA  
AAAAATATTTTGCCTACATTAAATTACCTTTATTGAAAGCAATGATTTTACATCAAT  
TTTATAATTTTCCCCGATGCAAAACTTGGCTATGAAGTTATTGAAAACCTAAAGAATTAA  
TGCTTATAAAAATACAATGAAATTATTCAATAAAAGAATTACTCAGGCGTCTATATGTG  
GCAATTGCTATTGCATTAGGATATAAAACCATCTATTATGTGGCATTGATTTTATGAAGGAG  
ATGTTATTATCCTTTGAAGCTATGAGTACAAATATAAAACAATCTTCTGGAAATAAAGA  
TTCAACCTCAATTGTCTAAGGAATACGATATAGAAGCATTAAATGTTAAAATCA  
ATATACAAAGTTAATATCTACGCATTGTGTGATGATTCTATTGGCAAATCATTCTTAT  
CAATTAATATTAAATAACAATTCACTTTAGAAAATAAGCATAATAATTCTATAATGATATT  
ATTGACTGATAATACTCCTGGCGTAAGTTTATAAAAATCAACTTAAAGCTGATAATAAATT  
ATGCTTAATTTATAATATTCTCATTCTAAAGATAATTAAATTAAACAAAGAAA  
TTGCGGTATTAACAAACCAACTCAACGAGCTAAAGCAAGAATCCAAAACCATCTATCCTA  
TAAACTAGGACAAGCTCATCACCACCATCACCACATGA
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Figure 2: Amino acid sequence of the fusion protein in CST-02.

MEKQNIAVILARQNSKGLPLKNLRKMNGISLLGHTINAAISSKCFDRIIVSTDGGGLIAEEAKNF
GVEVVLRPAELASDTASSISGVVIHALETIGNSGTVTLLQPTSPRLTGAHIREAFSLFDEKIKG
SVVSACPMEEHHPLKTLQINNGEYAPMRHLSDEQPRQLPQAFRPNGAIYINDTASLIANNCF
FIAPTKLYIMSHQDSIDIDTELDLQQAENILNHKESGGGILTRTRMENELIVSKNMQNIIAGN
GPSLKNINYKRLPREYDVRCNQFYFEDKYYLGKKIKAVFFNPVGFLQQYHTAKQLILKNEYEI
KNIFCSTFNLPFIESNDFLHQFYNFDPDAKLGYEVIENLKEFYAYIKYNEIYFNKRITSGVYMC
AIAIALGYKTIYLCGIDFYEGDVIYPFEAMSTNIKTIFPGIKDFKPSNCHSKEYDIEALKLLKS
IYKVNIYALCDDSI LANHFPLSININNNFTLENKHNN SINDILLTDNTPGVSFYKNQLKADNKI
MLNFYNILHSKDNLIKFLNKEIAVLKKQTQRACKARIQNHLSYKLGQAHHHHH